[6] Madsen (private communication) found that Mg ions lower the $K_m$ for 5'-AMP without affecting the $V_{max}$. Owing to this effect, the inhibition by ATP is markedly diminished in the presence of this metal ion. Similarly, the effect of 5'-AMP on the interaction of phosphorylase with its substrates could be modified by the presence of Mg ions and result in different numerical values for kinetic parameters. In previous papers[2, 4] an auxiliary enzyme system was used to measure the rate of the phosphorylase reaction and this necessitated the addition of Mg ions. It should therefore be emphasized that the present experiments were conducted in the absence of Mg ions.

[7] Cori, C. F., G. T. Cori, and A. A. Green, *J. Biol. Chem.*, **151**, 39 (1943).

[8] Katz, J., and W. Z. Hassid, *Arch. Biochem. Biophys.*, **30**, 272 (1951).

[9] The $K_m$ of glucose-1-P for phosphorylase *a* has been redetermined at saturating concentrations of glycogen and in the presence of $1 \times 10^{-3}$ *M* 5'-AMP. At pH 6.8 at 31° in 0.04 *M* Na-citrate-0.004 *M* 2-mercaptoethanol buffer a value of $4.8 \times 10^{-3}$ *M* was found. Madsen[3] reported a value of $5.1 \times 10^{-3}$ *M* for phosphorylase *b* at pH 6.7 and 30°.

[10] Madsen, N. B., and C. F. Cori, *Biochim. Biophys. Acta*, **15**, 516 (1954).

[11] We are indebted to Dr. Monod for sending us an unpublished manuscript entitled "Un modèle plausible de la transition allostérique."

[12] Wang, J. H., and D. J. Graves, *J. Biol. Chem.*, **238**, 2386 (1963); and *Abstracts*, 146th National Meeting, American Chemical Society, Denver, Colorado, January 1964, p. 32A.

[13] Levy, H. M., N. Sharon, and D. E. Koshland, Jr., these PROCEEDINGS, **45**, 785 (1959).

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## *EXPERIMENTAL PRODUCTION OF TESTICULAR TERATOMAS IN MICE**

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Testicular teratomas are rare in mice except in the inbred strain 129. Two per cent of the males of some sublines of this strain develop spontaneous teratomas congenitally.[1, 2] There is much evidence that these tumors are derived from primordial germ cells,[3] and it is known that the teratocarcinogenetic process is initiated before 15 days and not later than 17 days of gestation.

The incidence of spontaneous testicular teratomas in strain 129 is strongly influenced by environmental and genetic factors.[4] They occur more frequently in the left than the right testis and they are twice as frequent in second and later litters as in first. Susceptibility to teratocarcinogenesis is probably determined by multiple genes, but animals carrying a single gene, Steel ($Sl^J$), have twice as many tumors as their non-Steel littermates.

Testicular teratomas can be induced in fowl by intratesticular injection of toxic salts and hormones (see Guthrie[5]). Bresler[6] obtained two testicular teratomas in adult mice injected intratesticularly with copper sulphate and testosterone propionate. This observation is noteworthy in view of the fact that all of the teratomas in genetically susceptible strain 129 mice arise prenatally. This means that teratomas in mice may originate from either primordial germ cells (as they do in strain 129) or probably from spermatogonia in adults.

The original aim of this investigation was to find out if teratomas would arise in testes derived from genital ridges transplanted to adult spleens and testes. If so, would the graft site influence the pattern of differentiation of tissues in tera-

tomas? Unexpectedly, we found that grafting the $12^1/_2$-day genital ridge to the testis remarkably augments the incidence of teratomas. The original aim of this investigation was amplified to attempt to define the conditions underlying this new phenomenon.

*Materials and Methods.—Mice:* Most of the animals used in this investigation were from a stock of mice congenic with inbred strain 129/Sv. This stock was developed by introducing the genes *C*, *P*, and $Sl^J$ into the strain 129 genome, and is characterized by its relatively high incidence of testicular teratomas. About 10% of males in second and later litters carrying $Sl^J$ develop congenital teratomas.[4] $Sl^J$ is a spontaneous recurrence of *Sl* found in the C3H/Hu strain by K. P. Hummel.

*Grafting procedure:* The genital ridges were removed from fetuses 12 and 13 days after the mothers were found with mating plugs. Hosts ranged in age from about 28 days to fully mature males. The host testes were exposed through a median ventral incision in the skin and body wall. The spleen was exposed through a lateral incision. The grafts were drawn into and expelled from a micropipette attached to rubber tubing with a mouthpiece. Most grafts were removed after 7, 13, or 21 days, fixed in Vandegrift's solution, serially sectioned at 7 $\mu$, and stained with hematoxylin and eosin. A few grafts were allowed to grow for longer periods.

Approximately half of the grafts developed into ovaries and were not included in any of the results presented here.

*Results.*—The size of 31 spontaneous testicular teratomas was measured in mice 15 days of gestation to birth by counting the number of serial histological sections that contained each tumor. Size was plotted against age in days. The intersection of this curve and the base line was calculated to be 11.8 days (Fig. 1). Judging from this, it was estimated that teratocarcinogenesis may be initiated at about 12 days of gestation. Another growth curve was prepared using the greatest diameter of the same tumors as an index of size and was identical to that shown in Figure 1.

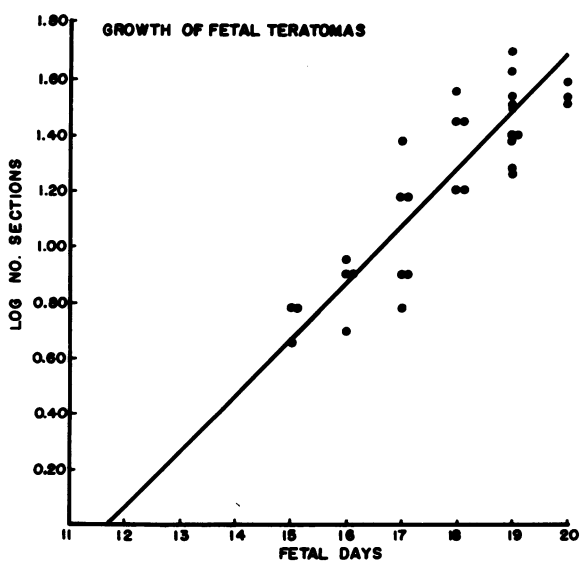


FIG. 1.—Growth curve of testicular teratomas in fetal mice from 15 days' gestation to birth. Size was measured by number of consecutive serial sections containing each tumor.

*Influence of various factors on teratocarcinogenesis:* (1) *Age of genital ridge and site of graft:* Eighty-two per cent of $12^1/_2$-day genital ridges of strain 129/Sv-$Sl^J$ male fetuses grafted to the testes of adult males of the same strain developed testicular teratomas. Nine per cent of $12^1/_2$-day 129/Sv-$Sl^J$ genital ridges developed teratomas when grafted to the spleen. Eight per cent of $13^1/_2$-day 129/Sv-$Sl^J$ genital ridges developed teratomas when grafted to the testes of adult males (see Table 1). Teratocarcinogenesis in grafted genital ridges is strongly influenced by the graft site. This process is also markedly affected by the age of the graft.

TABLE 1

EFFECT OF AGE OF GENITAL RIDGE AND SITE OF GRAFT ON TERATOCARCINOGENESIS IN STRAIN 129/Sv-$Sl^J$ MICE

| | $12^1/_2$-Day | | $13^1/_2$-Day | |
|---|---|---|---|---|
| Graft site | No. | % Teratomas | No. | % Teratomas |
| Testis | 61 | 82 | 32 | 8 |
| Spleen | 92 | 9 | — | — |

TABLE 2

EFFECT OF LITERALITY AND LITTER SERIATION ON INCIDENCE OF TERATOMAS IN GENITAL RIDGE GRAFTS

| Origin of genital ridge | No. of teratomas | % Teratomas |
|---|---|---|
| Left | 20 | 87 |
| Right | 17 | 81 |
| 1st litter | 17 | 81 |
| 2nd litter | 23 | 82 |

(2) *Laterality and litter seriation:* Spontaneous teratomas in strain 129 mice occur about twice as frequently in the left as in the right testis. The incidence of tumors is also doubled in animals in second and later litters compared with those in first litters. These laterality and litter seriation differences in incidence are not apparent in teratomas that develop from $12^1/_2$-day male genital ridges grafted to the testes of adults (see Table 2).

(3) *Host genotype:* In order to find out if the enhancing influence of the host is specific for males of the Steel subline, $12^1/_2$-day 129/Sv-$Sl^J$ genital ridges were grafted intratesticularly to other sublines of strain 129 and to strain AL/Ks males. Sublines 129/Re and 129/Sv have a strong enhancing effect on teratocarcinogenesis (Table 3). Furthermore, about half of the testes derived from $12^1/_2$-day genital ridges of strain 129/Sv-$Sl^J$ fetuses developed teratomas after transplantation to testes of strain AL/Ks. These results demonstrate that the enhancement of teratocarcinogenesis in developing strain 129 testes is not specific for adult testes of this strain. It appears, however, that adult testes of strain 129/Sv-$Sl^J$ exert a stronger influence than those of other sublines of strain 129 and strain AL/Ks.

TABLE 3

HOST INFLUENCE ON TERATOCARCINOGENESIS IN GRAFTS OF $12^1/_2$-DAY 129/Sv-$Sl^J$ GENITAL RIDGES

| Host | No. grafts | % Teratomas |
|---|---|---|
| 129/Sv-$Sl^J$ | 61 | 82 |
| 129/Re | 27 | 67 |
| 129/Sv | 29 | 66 |
| AL/Ks | 12 | 41 |

TABLE 4

INFLUENCE OF DONOR GENOTYPE ON TERATOCARCINOGENESIS

| | Host | | |
|---|---|---|---|
| Donor | 129 | AL/Ks | % Teratomas |
| AL/Ks | 22 | 7 | 0 |
| DBA/1 | 11 | | 0 |
| C3H/Hu | 5 | | 0 |

(4) *Donor genotype:* Forty-five genital ridges from fetuses of strains AL/Ks, DBA/1, and C3H mice were grafted to adult testes (Table 4). The mice designated as strain 129 in Table 4 include 17 129/Sv-$Sl^J$ and 21 mice of other sublines of strain 129. All grafts were recovered about 10 days after transplantation so that they were not destroyed by a homograft reaction. None had teratomas. The genotype of the graft determines the susceptibility to teratocarcinogenesis.

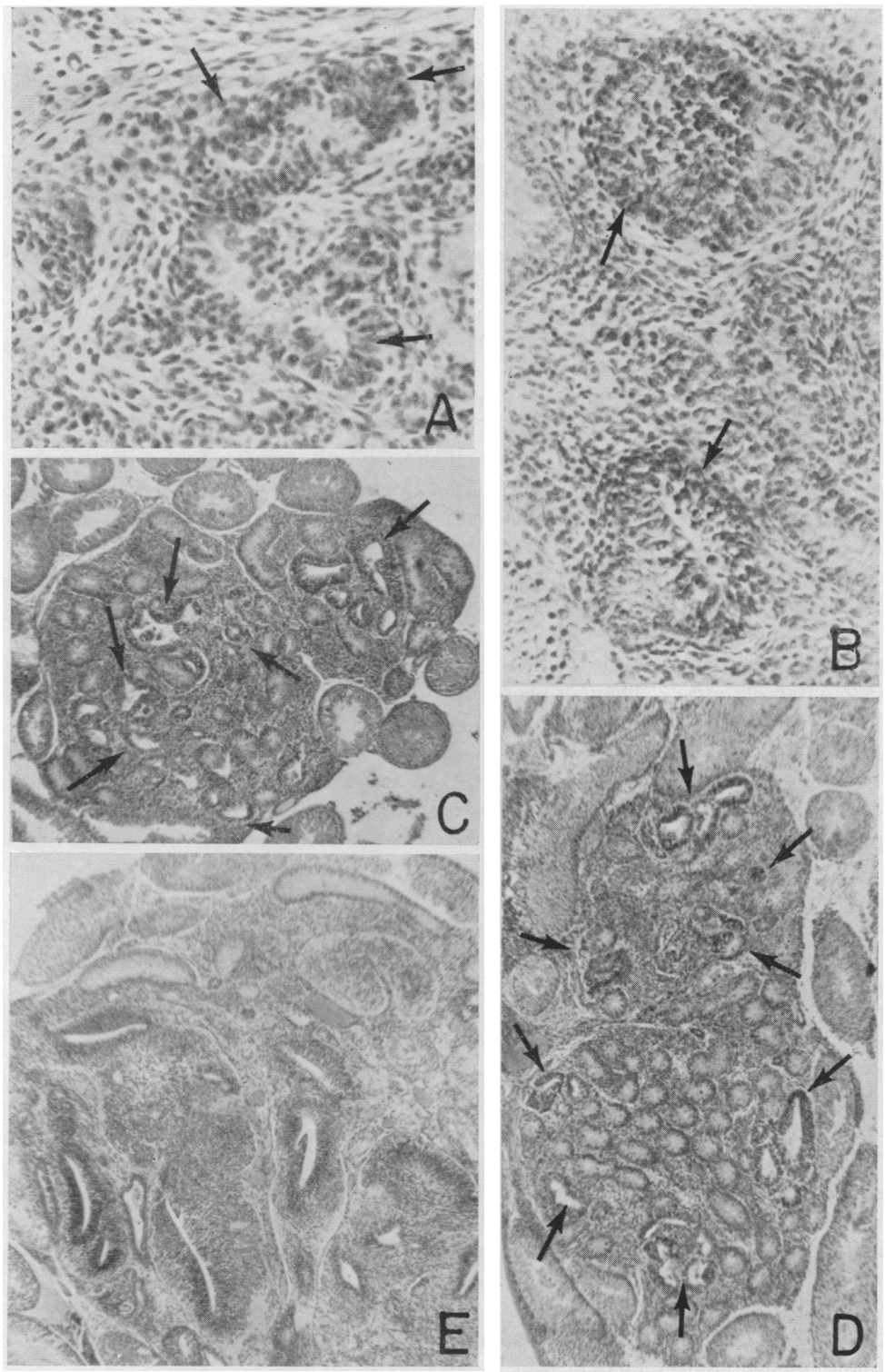


FIG. 2.—Teratomatous foci in testes derived from 12½-day genital ridge grafted to adult testes. *A:* LS7671 Three minute foci (arrows) within seminiferous tubules derived from a 7-day graft. *B:* LS7783 Two foci enlarging seminiferous tubules in a 9-day graft. *C:* LS7458 Six foci in a 13-day graft. *D:* LS7463 Eight foci in a 13-day graft. *E:* LS7707 Twenty-one-day graft composed mostly of immature and adult neural tissue.

*Histology of teratomas developing from intratesticular grafts of 12½-day genital ridges:* In general the teratomas resulting from grafting 12½-day genital ridges to the testes of adult mice were similar in histologic makeup to those that develop spontaneously in strain 129 mice. There was a conspicuous difference, however, in the number of foci. In testes of fetal mice containing spontaneous teratomas, 1–6 foci were observed, and in testes with multiple foci the teratomas usually occurred within the same tubule near each other.[2] It appeared as if the several tumors within the same testes were descended from a common source. In contrast, most of the tumors developing in grafted genital ridges had many (from 10+ to 20+) foci widely separated from each other, and they obviously originated from many cells (Fig. 2*C* and *D*). The tubules derived from the grafts were easily distinguished from the seminiferous tubules of the host. They usually formed a discrete mass enveloped by a tunica.

Many of the tumors observed seven and nine days after grafting were intratubular and resembled spontaneous tumors in 15–17 day fetuses (Fig. 2*A* and *B*). Small tumors were always completely enclosed in the seminiferous tubules, as were small tumors that developed spontaneously in fetal mice. Larger tumors were always continuous with seminiferous epithelium as are spontaneous teratomas in fetal mice. These early tumors were composed of undifferentiated embryonal cells.

The tumors which developed in grafts of genital ridges observed 13 days after transplantation were larger than the above and resembled spontaneous tumors in 2–5-day-old mice. They also contained many undifferentiated embryonal cells, but also had ectodermal and endodermal epithelium and immature neuroepithelium (Fig. 2*C* and *D*).

Teratomas examined approximately three weeks after grafting genital ridges were composed of a wide variety of tissues but immature and adult neural tissue predominated (Fig. 2*E*).

We have examined a limited number of teratomas derived from genital ridges grafted for over four weeks. Some were comparatively small—approximately double the diameter of the normal testis. These contained mature tissues (Fig. 3*A*), principally neural. Others greatly enlarged the testis and contained immature proliferating tissues and few undifferentiated embryonal cells. These tumors were larger than most spontaneous teratomas. Probably most of the increase in size of the tumors derived from grafted genital ridges may be ascribed to the fact that they are composed of many foci. Many tissue types were represented in these tumors.

*Histology of teratomas developing from intrasplenic grafts of 12½-day genital ridges:* Only eight of 92 genital ridges grafted to the spleen developed teratomas (Table 1). These tumors were smaller than their intratesticular counterparts and most were simple in composition, containing mostly adult neural tissue with a few cysts lined with ciliated epithelium (Fig. 3*B*). There was a striking difference between tumors in the two graft sites regarding degree of differentiation. The tumors in intratesticular growths examined 13 days after grafting contained undifferentiated embryonal cells and immature tissues with dividing cells as well as mature tissues. The intrasplenic tumors, on the other hand, contained more mature tissues with fewer immature cells than in intratesticular tumors.

The tumor cells which develop in intratesticular grafts proliferate for a longer period than in intrasplenic grafts. It appears that the spleen provides a less

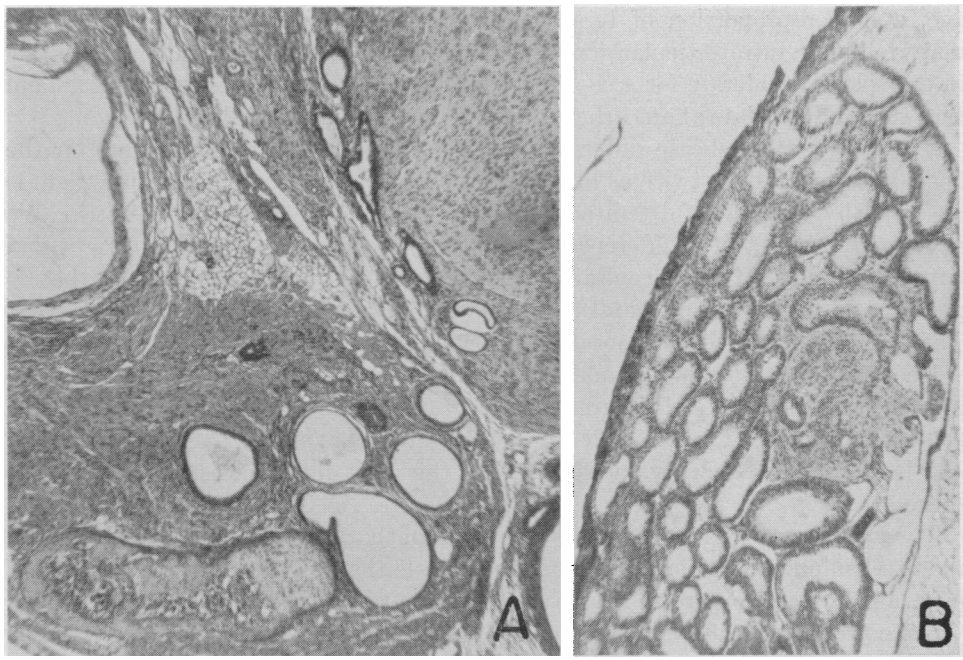


FIG. 3.—Teratomas in testes derived from $12^1/_2$-day genital ridges grafted intratesticularly (*A*) and intrasplenically (*B*). *A:* LS8144 Intratesticular graft composed of many types of tissues including cartilage and bone with marrow (bottom), striated muscle (center), and neural tissue (upper right). Adipose tissue and epithelium are also present. *B:* LS7136 Twenty-one-day intrasplenic graft containing a small teratoma composed of adult neural tissue and ciliated epithelium.

favorable environment for the growth of these cells during early stages of development of the tumors. Alternatively, the splenic environment promotes the differentiation of the undifferentiated embryonal cells which are the stem cells of these tumors.

*Discussion.*—We have previously emphasized the importance of genetic and environmental factors on teratocarcinogenesis in strain 129 mice. Several genes are involved, but animals carrying a single gene, Steel, have twice as many teratomas as their non-Steel littermates. There is a maternal influence on the development of these tumors; animals in second and later litters have about twice as many teratomas as animals in first litters. Local environmental influences are involved; twice as many teratomas are found in left as in right testes. To these influences, we now add another, the strong teratocarcinogenetic effect exerted by the environment of the adult testis on the $12^1/_2$-day genital ridge. Approximately 10 times as many testes derived from $12^1/_2$-day genital ridges grafted to the adult testes had tumors as those grafted to the spleen. In addition to the increased proportion of intratesticular grafts which contained tumors, there were many more independent foci per testis than in intrasplenic grafts or in teratomas which develop spontaneously.

The site of the genital ridge graft influences the initiation of teratocarcinogenesis, and also the pattern of growth. Tumors in grafts to the spleen are smaller and simpler in composition than those in grafts to the testis. In tumors of comparable

age, a higher proportion of teratomatous tissues in the intrasplenic grafts are mature than in intratesticular grafts. It appears as if the cells in tumors initiating in the spleen proliferate for a shorter time than those in intratesticular grafts and that they differentiate at an earlier stage to become adultlike cells.

Apparently the delicate difference in the environment which accounts for the left testis being about twice as likely to develop spontaneous tumors as the right is obscured by the powerful teratocarcinogenetic influence of the adult testis. We were unable to detect a difference in tumor-incidence between grafts of right and left genital ridges. Both genital ridges are equally susceptible, as would be expected, and local environmental influences account for the difference in incidence. The maternal influence on tumor incidence is similarly masked.

The genotype of the adult male carrying grafts of genital ridges influences the incidence of teratomas which are initiated. Animals from the Steel subline exert a stronger influence than animals from other sublines of strain 129 and from strain AL/Ks. It is noteworthy, however, that this host influence is not strain 129-specific.

The genotype of the graft is more critical than that of the donor. Animals from the Steel subline are genetically more susceptible than other sublines of strain 129 mice, and grafts from foreign strains are not susceptible to this influence.

Since the tumors can be recognized one week after grafting, we concentrated our efforts on early stages at the expense of observing long-term grafts. However, we have observed a few cases 44 days after transplantation, and the tumors are much larger than most of those which develop spontaneously. They were composed mostly of differentiated tissues, and we tentatively suggest that the increased size may be attributed to the larger number of independently developing foci in the experimentally produced tumors as compared to those developing spontaneously.

Teratocarcinogenesis is initiated during a sharply defined prenatal period. We have previously estimated that they arise shortly before 15 days and not later than 17 days of gestation. The results presented here demonstrate that there is a sharp decline in susceptibility between $12^1/_2$ and $13^1/_2$ days of gestation. Apparently there is a developmental process occurring during this period which drastically alters the susceptibility of the primordial germ cell to teratocarcinogenesis. We are continuing to investigate this new phenomenon.

*Summary.*—Eighty-two per cent of $12^1/_2$-day genital ridges from strain 129 fetuses developed testicular teratomas when transplanted to adult testes. This is a much higher incidence than that which occurs spontaneously. This effect was not observed in testes derived from $12^1/_2$-day genital ridges grafted to spleens of adults nor in intratesticular $13^1/_2$-day genital ridges. The site of origin of teratomas and the stage of development of primordial germ cells from which these tumors are derived play strong roles in teratocarcinogenesis. The primordial germ cell passes through a developmental stage from $12^1/_2$ to $13^1/_2$ days of fetal life which markedly alters its susceptibility to this process.

Genital ridges of sublines of strain 129 are susceptible to varying degrees, but genital ridges from other strains do not develop teratomas under these conditions. The genotype of the host also plays a role, but it is weak. Strain 129 genital ridges develop teratomas when grafted to the testes of foreign strains. This model will be used to investigate various factors involved in teratocarcinogenesis in mice.

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[1] Stevens, L. C., and C. C. Little, these PROCEEDINGS, **40**, 1080 (1954).

[2] Stevens, L. C., *J. Natl. Cancer Inst.*, **28**, 247 (1962).

[3] Stevens, L. C., *Année. Biol.*, Ser. 4, **1**, 585 (1962).

[4] Stevens, L. C., and J. A. Mackensen, *J. Natl. Cancer Inst.*, **27**, 443 (1961).

[5] Guthrie, J., *Brit. J. Cancer*, **18**, 130 (1964).

[6] Bresler, V. M., *Probl. Oncol.*, **5**, 24 (1959).

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## *ACATALASEMIC MICE**

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A great many genetically determined inborn errors of metabolism are known in the human population, and the list is constantly growing. Few counterparts, however, are available for the more detailed studies that can be carried out in experimental animals, and none, to our knowledge, has ever been deliberately and successfully sought.

The work of Takahara in Japan[1] and later of Aebi in Switzerland[2] has demonstrated that it is possible for a healthy human being to be essentially devoid of blood catalase. These findings suggested to one of us (R. N. F.) that it might be feasible to obtain an acatalasemic strain of animals, which could then be used in studying certain radiation, oncological, and other problems. Whether such an animal would necessarily be also acatalatic—i.e., free of catalase in all tissues—could not be stated and still cannot. However, agents are available, of which 3-amino-1,2,4-triazole is the best known but not the most effective,[3] which, with a fair degree of specificity, will inactivate *in vivo* the catalase of the solid tissues, though not of the erythrocytes. Thus if an acatalasemic animal is obtained, presumably he could readily be rendered acatalatic.

We now wish to report that we have produced genetically acatalasemic mice. Because at this stage we are using only a nondestructive blood assay, we are not yet able to make any statements as to the catalase activity of other tissues.

One of us (W. L. R.) has for some time[4] been obtaining radiation-induced mutations at specific loci in mice. Two of the current experiments set up for this purpose were chosen as potential sources of a mutation to acatalasemia. In both experiments, the offspring of the irradiated fathers, who had received a total dose of 600 r in fractionated exposures, were showing a high frequency of specific locus mutations. These mice, after scrutiny at Oak Ridge for the specific locus mutations, have been examined at Argonne by a recently described, rapid, semiquantitative screening test for blood catalase.[5] Approximately 12,000 mice have been examined, and two with low blood catalase are clearly genetic mutants for this defect. Several other